Vitamin D in polycystic ovary syndrome: Relationship to obesity and insulin resistance

Joham, AE, Teede, HJ, Cassar, Samantha, Stepto, Nigel, Strauss, Boyd J, Harrison, CL, Boyle, J and de Courten, Barbora (2016) Vitamin D in polycystic ovary syndrome: Relationship to obesity and insulin resistance. Molecular Nutrition and Food Research, 60 (1). 110 - 118. ISSN 1613-4125

Vitamin D in Polycystic Ovary Syndrome: Relationship to Obesity and Insulin Resistance

Short title: Vitamin D, obesity and insulin resistance in PCOS

Anju E Joham[1,2], Helena J Teede[1,2], Samantha Cassar[3], Nigel K Stepto[3], Boyd J Strauss[4], Cheryce L Harrison[1], Jacqueline Boyle[1], Barbora de Courten [1,2]

1. Monash Centre for Health Research and Implementation, School of Public Health and Preventative Medicine, Monash University, Locked Bag 29, Clayton, VIC 3168, Australia

2. Diabetes and Vascular Medicine Unit, Monash Health, Locked Bag 29, Clayton, VIC 3168, Australia

3. Institute of Sport Exercise and Active Living, Victoria University, Melbourne, Australia

4. Department of Medicine, School of Clinical Sciences, Monash University, Melbourne, Australia

Correspondence:

A/Prof Barbora de Courten, MD PhD FRACP MPH

Monash Centre for Health Research and Implementation, School of Public Health and Preventive Medicine, Monash University

Locked bag 29, Monash Medical Centre

Clayton, Victoria 3168, Australia

Telephone: + 613 9594 7545

Fax: + 613 9594 7550

Email: barbora.decourten@monash.edu

Key words: Vitamin D, insulin resistance, obesity, polycystic ovary syndrome, PCOS

Word count: 3418

*Conflict of interest***:** The authors have nothing to disclose.

Abstract

Scope

Polycystic Ovary Syndrome (PCOS) is underpinned by IR. In PCOS, the relationships between vitamin D, adiposity and IR are unclear. We aim to explore these relationships in lean and overweight women with PCOS.

Methods and results

This is a cross-sectional study conducted in a tertiary medical centre. Participants included 42 women with PCOS and 34 controls without PCOS. Vitamin D and metabolic markers were measured. Detailed body composition and gold standard hyperinsulinaemic euglycaemic clamps were performed. The main outcome measures were plasma levels of vitamin D, adiposity measures and glucose infusion rate (GIR).

Vitamin D levels were lower in overweight women with PCOS compared with overweight controls (31.6 and 46.1 nmol/L respectively, p=0.01). Vitamin D was not associated with IR after adjustment for confounders; however, there was a significant interaction between PCOS and % body fat. Further analysis by PCOS status revealed that vitamin D was associated with IR in the PCOS group (β coefficient 2.1, 95% CI 0.2–4.0, p=0.03), but not in the non-PCOS group.

Conclusion

Vitamin D is associated with IR in women with PCOS, but not in controls. Large intervention studies are needed to determine if vitamin D supplementation can improve IR in PCOS.

Introduction

Vitamin D is a fat soluble vitamin that is involved in bone metabolism and calcium homeostasis (1). It has been emerging over recent years that vitamin D may have a role in disorders outside the skeletal system including immune disorders, diabetes, hypertension, cardiovascular disease, infectious diseases and cancer (2-4). Vitamin D deficiency is very prevalent worldwide, as people adopt sedentary indoor lifestyles and use sunscreen and protective clothing to reduce skin cancer risk (5). Despite our sunny climate, vitamin D deficiency is also common in Australia. The AusDiab Study reported vitamin D deficiency (vitamin D <50 nmol/L) in 50% of women and 31% of men living at latitudes >35 degrees south – which includes Melbourne, the location of the current study (6). Low vitamin D levels have been found in type 2 diabetes mellitus (T2DM) and correlate with obesity, pancreatic beta cell dysfunction and insulin resistance (IR) and risk of developing T2DM (7-10). Supplementation of vitamin D has been shown to improve IR (11) and prevent T2DM (9).

Polycystic ovary syndrome (PCOS) is a common disorder that affects 9-21% of reproductive-aged women (12), depending on the population studied and the diagnostic criteria applied. PCOS diagnosis is based on menstrual disturbance (oligo or amenorrhoea), clinical or biochemical hyperandrogenism and polycystic ovaries on ultrasound (13). IR plays a central pathophysiological role in the majority of women with PCOS (14) and underpins significant metabolic complications in PCOS including dyslipidaemia, dysglycaemia and hyperandrogenism (15). IR in PCOS is both independent of and exacerbated by obesity (16). Approximately 60% of Australian women with PCOS are overweight or obese and women with PCOS may also have a relatively greater central adiposity distribution compared to controls with equivalent BMI (16).

Several recent studies have explored the association of vitamin D with IR and metabolic features in PCOS (17-19); however these studies used indirect measures of IR such as oral glucose tolerance tests, Homeostasis Model Assessment (HOMA) and Quantitative Insulin Sensitivity Check Index (QUICKI). There has only been one study to date examining the relationship between vitamin D and IR in PCOS using hyperinsulinaemic euglycaemic clamps (gold standard); however this study did not have a control population. This study suggested that vitamin D levels in PCOS are primarily related to adiposity (20). A recent randomised controlled trial shows that supplementation with vitamin D improved IR, suggesting that vitamin D might have a protective role in the development of IR in women with PCOS (21). Women with PCOS often demonstrate clustering of cardiovascular risk factors and may have an increased risk of cardiovascular disease (22). It has been suggested that vitamin D may have a protective effect on some cardiovascular risk factors (23).

To this end, we measured adiposity, IR (gold standard measures), lipid profile and blood pressure in lean and overweight women with PCOS and without PCOS to further explore relationships between vitamin D, metabolic and cardiovascular risk factors in PCOS. The current study adds to existing literature with the inclusion of both women with PCOS and controls, including lean and overweight subjects and the use of clamp studies and detailed body composition measures. We hypothesized that obese women will have lower vitamin D compared to lean women and women with PCOS will have lower vitamin D levels compared to women without PCOS. In addition, we hypothesized that vitamin D will be further lowered by presence of cardiovascular risk factors.

Methods and methods

Subjects

Women were recruited from community advertisements (16). This vitamin D study is part of a PCOS study investigating insulin resistance in PCOS (16). 76 women were eligible and had completed 3 months run-in phase as previously described (16). The women were categorised according to PCOS status and BMI. Women were allocated into two BMI categories (lean and overweight) based on the threshold BMI of 27kg.m^{-2}, as an *a priori* decision, as this is the inflexion point in the relationship between BMI and IR (16). We analysed baseline data from 76 women (n=22 lean women and n=20 overweight women with PCOS and n=19 lean and n=15 overweight control women without PCOS).

As previously described, diagnosis of PCOS was undertaken by expert endocrinologists based on Rotterdam criteria with two of a) irregular menstrual cycles (<21 or >35 days), b) clinical (hirsutism, acne) or biochemical (elevation of at least one circulating ovarian androgen) hyperandrogenism and c) PCO on ultrasound (24). Hyperprolactinemia, thyroid dysfunction and specific adrenal disorders were excluded clinically and where indicated, biochemically. All women without PCOS had regular menses and no evidence of clinical or biochemical hyperandrogenism. Exclusion criteria were smoking, diabetes, recent weight change of 5 kilograms or more in the previous six months, actively trying to lose weight and pregnancy.

The Southern Health Research Advisory and Ethics Committee approved the study and all participants gave written informed consent.

Study Design

At screening (3 months prior to baseline), standard diet and lifestyle advice were delivered [Heart Foundation of Australia recommendations (www.heartfoundation.org.au)] as previously described (25). Medications affecting end-points including insulin sensitisers, anti-androgens and hormonal contraceptives were ceased for 3 months. Data were collected following this 3 month run-in phase and was collected in the follicular phase of the menstrual cycle wherever feasible.

Anthropometric Measurements

Participants were weighed lightly clothed without shoes (Tanita TBF310, Tokyo, Japan) followed by height measurements (Stadiometer Holtain, Wales, UK) for the calculation of BMI [weight (kilogram) / height squared (metre^2)]. Waist circumference (WC) was measured at the umbilicus by an experienced operator.

Body Composition and Adipose Tissue Distribution

Fat mass, abdominal fat mass and fat-free mass (FFM) were measured by dual-energy x-ray absorptiometry (DEXA) as described elsewhere (25). Single-slice Computer Tomography (CT) images were acquired at the level of L4–L5 intervertebral disc space and abdominal visceral fat (AVF) and abdominal subcutaneous fat cross-sectional areas (centimeters squared) were calculated as previously described (25).

Hyperinsulinaemic euglycaemic Clamp

IR was measured using the hyperinsulinaemic euglycaemic clamp technique as previously described (25, 26). Fasting venous blood samples were collected, centrifuged and stored for assessment of glucose, insulin, vitamin D, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL) and triglycerides as previously described (25). The glucose

infusion rates (GIR) were calculated during the last 30 minutes of the hyperinsulinaemic euglycaemic clamp and expressed as glucose (mL) per hour per body surface area (m^2**)** per minute.

Plasma vitamin D

Plasma vitamin D was determined using Liaison 25 OH vitamin D assay, a commercial direct competitive chemiluminescent immunoassay (CLIA) on a single assay. Vitamin D sufficiency is defined as plasma 25(OH)D concentration above 75 nmol/L. Vitamin D insufficiency refers to levels between 50 to 75 nmol/L and deficiency refers to levels less than 50 nmol/L (27).

Metabolic syndrome

Metabolic syndrome was determined using the World Health Organization (WHO) criteria which requires the presence of IR in addition to at least two other criteria (28). However, we did not have any information regarding urine albumin excretion, which is one of the additional criteria.

Statistics

All data were analysed using STATA software version 11.0 (StataCorp, TX). Data were assessed for normality and log transformed where appropriate and analysed using univariate analysis of variance (ANOVA). Post hoc analysis was conducted adjusting for multiple comparisons using the appropriate method (tukey) to adjust for possible type 1 errors. Continuous data are presented as mean ± SD or median (interquartile range) as appropriate. Correlation of vitamin D with IR, adiposity and cardiovascular risk factors was assessed

using Pearson or Spearman rank correlation where appropriate. Linear regression was used for assessment of factors associated with plasma vitamin D levels. Statistical significance was set at α level of $p < 0.05$.

Results

Results are presented for 76 subjects with samples available for analysis including vitamin D. 22 lean women and 20 overweight women with PCOS and 19 lean and 15 overweight control women without PCOS were eligible at screening, completed the 3 month run-in phase with a steady diet, were compliant with withdrawal of relevant medications affecting glucose metabolism and completed data collection.

PCOS vs. non-PCOS women: cardiometabolic status and vitamin D levels

All baseline metabolic and clinical characteristics are presented in Tables 1 and 2. The overweight women with PCOS were younger than controls (29.8±5.5 years vs 35.1±4.1, p=0.004). There were no significant differences within the lean and obese groups with regard to blood pressure or lipid profile (Table 2).

Overall, 71% (30/42) of women with PCOS were vitamin D deficient compared to 56% (19/34) of women without PCOS. Vitamin D levels were higher in overweight control women compared with overweight women with PCOS before (46.1±20.0 vs 31.6±11.3 nmol/L respectively, p=0.01) and this persisted after adjustment for BMI and abdominal visceral fat (p=0.01). Vitamin D levels were similar between lean groups (p=0.97) (Table 2).

Vitamin D and obesity

The lean and overweight experimental groups had similar adiposity within groups as determined by BMI and visceral abdominal fat content determined by CT (Table 2). Vitamin D was negatively associated with waist circumference (r= -0.41, p<0.001), BMI (r= -0.36, p=0.002), total fat mass (r= -0.39, p=0.001), % body fat (r= -0.37, p=0.002) and abdominal visceral fat (r= -0.41, p<0.001) before and after adjustment for age (all p<0.01). The correlation of vitamin D with adiposity measures between PCOS and non-PCOS groups are presented in Figure 1.

Vitamin D and insulin resistance

IR was not different between the lean PCOS and overweight controls as previously reported (16). As expected, IR was greatest in the overweight PCOS cohort and lowest in the lean PCOS cohort (Table 2). Vitamin D levels were positively associated with GIR (r=0.31, p=0.01). Vitamin D was associated with IR (GIR) on univariate regression (β coefficient 1.5, 95% CI 0.4 – 2.6, p=0.01), but was not associated with IR on multivariate regression when adjusted for PCOS status, age and % body fat. Both PCOS and % body fat were associated with IR in the multivariable regression model (β coefficient -66.3, 95% CI -104.5 - -28.2, p=0.001 and β coefficient -3.7, 95% CI -5.6 - -1.8, p<0.001) (Table 3). To adjust for seasonal effects, the month of the year when clamp data were collected was included in the multivariable regression model, with no significant effect on results. There was a significant interaction between PCOS and % body fat for the outcome of IR (p=0.003). Further analysis by PCOS status revealed that vitamin D was associated with IR, independent of % body fat in the PCOS group (β coefficient 2.1, 95% CI 0.2 – 4.0, p=0.03). Vitamin D was not associated with IR in the non-PCOS group (Table 4).

Vitamin D and CVD risk factors

There was a relationship between vitamin D and triglycerides after adjustment for age (p=0.01); however, this relationship was no longer significant after further adjustment for abdominal visceral fat (p=0.29). There was also a moderate correlation between vitamin D and high density lipoprotein (HDL) cholesterol before (r=0.38, p=0.001) and after adjustment for age and % body fat (p=0.001 and p=0.03 respectively). However, this relationship was no longer significant after further adjustment for abdominal visceral adiposity (p=0.23). There was no correlation with vitamin D and total cholesterol or LDL cholesterol levels. There was no correlation between vitamin D and systolic or diastolic blood pressure.

Vitamin D and metabolic syndrome

Ten of the 42 PCOS participants (23.8%) and three of the 33 control participants (9.1%) fulfilled the WHO diagnostic criteria for metabolic syndrome. Within the overweight cohort, ten of the 20 PCOS participants (50%) and two of the 14 control participants (14.3%) had metabolic syndrome. Vitamin D levels were lower in participants with both PCOS and metabolic syndrome compared to those with PCOS and without metabolic syndrome (27.4±9.8 vs 45.4±16.0 nmol/L respectively, p=0.002). A greater number of features of metabolic syndrome also correlated to lower vitamin D levels (Figure 2).

Discussion

In the present study, we report that despite similar adiposity, vitamin D levels were lower in overweight women with PCOS compared with overweight controls. We also showed using gold standard techniques, that vitamin D was correlated with IR and adiposity measures. A further novel finding from this study is that vitamin D was associated with IR in the PCOS group, but not in the non-PCOS group. With regard to cardiovascular risk factors, vitamin D

levels correlated with triglyceride levels and HDL cholesterol, but not total cholesterol, LDL levels or blood pressure. Finally, metabolic syndrome in PCOS was associated with lower levels of vitamin D compared to PCOS without metabolic syndrome and the number of features of metabolic syndrome was negatively progressively associated with lower levels of vitamin D overall.

Vitamin D deficiency is common worldwide with estimated prevalence rates between 10 to 60%, although the definition of vitamin D deficiency remains controversial (29). It is estimated that 67-85% of women with PCOS are vitamin D deficient with levels less than 20 ng/mL (30), however reports are inconsistent. In the present study, we found that vitamin D was lower in overweight women with PCOS compared to overweight controls.

It is unclear if the association between vitamin D and PCOS is primarily related to PCOS status per se or primarily related to greater visceral adiposity or to the IR more often seen in women with PCOS (31). Several PCOS studies have reported an inverse relationship between vitamin D and BMI and waist hip ratio; however few studies have utilized gold standard measures of adiposity from DEXA and CT derived body fat (20, 32-34). Our current study uses detailed body composition measures with both DEXA and CT and confirms existing literature. Vitamin D levels appear to be 27-56% lower in obese women with PCOS compared with non-obese women with PCOS (30). Women with PCOS who have hirsutism may be less inclined to sun exposure. In the present study, vitamin D was 36% lower in overweight women with PCOS compared to lean controls. Given that vitamin D is a fat soluble vitamin, it has been hypothesized that there may be lower bioavailability due to greater adipose tissue sequestration of vitamin D in obese states (35). It is also possible that there may be dietary differences or variance in sunlight exposure between obese and non-

obese women as obese individuals are likely to be more sedentary hence spend more time indoors (30), with more research needed to explore mechanisms of low vitamin D in obesity.

It has been proposed that vitamin D deficiency may cause and worsen IR independent of adiposity (7, 36). The large, cross-sectional, nationally representative NHANES study (n=14,679) reported that IR determined by HOMA was inversely related to vitamin D after adjustment for confounders (8). Analysis of 808 non-diabetic participants of the Framingham Offspring Study found that plasma vitamin D levels were inversely associated with HOMA (7). Consistently, here we show that in women with PCOS, vitamin D is inversely associated with IR. Importantly, vitamin D supplementation (4000 IU/day) significantly improves IR (measured as HOMA) compared to placebo (11). A recent randomized double-blind placebo-controlled study by Asemi et al was conducted in 104 overweight and obese vitamin D deficient women diagnosed with PCOS. Participants were divided into 4 groups and received calcium and/or vitamin D supplementation or placebo, with vitamin D and calcium decreasing serum insulin levels and improving IR (21). This raises the potential for vitmain D therapy to improve IR in PCOS with further research needed.

IR is a key hormonal abnormality in women with PCOS; there is an extrinsic/obesity related IR and an intrinsic IR that is seen even in lean women with PCOS (16). It is unclear whether vitamin D deficiency and IR co-exist in PCOS women or whether the two are causally related. Several studies show an inverse association between vitamin D and IR in PCOS, using indirect measurements of IR (17-19). Muscogiuri et al (20) found that vitamin D deficiency was related to obesity and not IR using hyperinsulinaemic euglycaemic clamps and DEXA derived adiposity measures. However, only 37% of the 38 Italian women with PCOS were vitamin D deficient, potentially underestimating the relationship between vitamin D and IR. In addition, the Italian study did not have a control population. In the current study,

71% of the women with PCOS were vitamin D deficient and we also included women with more severe vitamin D deficiency. Gold standard hyperinsulinaemic euglycaemic clamps were used to measure IR with detailed body composition measures using both CT and DEXA and a control population of both lean and overweight women were studied. In the present study, we demonstrate that vitamin D was associated with IR independent of body fat percentage in the PCOS group, but not in the control group. A recent systematic review of 29 studies found that on linear multivariate analysis, serum vitamin D was not an independent predictor of IR in women with PCOS (37). However the review included many studies where the mean BMI of participants with PCOS ranged from 25.1 to 28.0 kg/m^2, which may have underestimated the relationship. Our study, with an overall mean BMI of 28.9 kg/m^2 in the PCOS group showed that vitamin D was independently associated with IR in women with PCOS. In addition, some the intervention studies detailed in the review included subjects who were only vitamin D insufficient but not deficient, had inadequate replacement of vitamin D and included studies with suboptimal study design.

There are several meta-analyses that support an inverse association between vitamin D levels and the presence of major cardiovascular risk factors including hypertension, diabetes and metabolic syndrome, though the effect of vitamin D supplementation on cardiovascular risk factors is not clear (38). Prior studies have also shown relationships between low vitamin D levels and cardiovascular risk factors in PCOS. Vitamin D deficiency has been associated with systolic and diastolic blood pressure (33) and also with total cholesterol, triglycerides (33) and lower HDL cholesterol (34) levels. In the present study, we show associations between vitamin D and HDL cholesterol and triglycerides but not total or LDL cholesterol. The association between vitamin D and both HDL and triglycerides persists after adjusting for % body fat, but the association was no longer significant after adjusting also for

abdominal visceral fat. This suggests that abdominal visceral adiposity may be driving the relationship between vitamin D and HDL and triglycerides and highlights the importance of measuring visceral adiposity when studying vitamin D in PCOS.

A large observational study of 206 women with PCOS revealed that women with both PCOS and metabolic syndrome had lower vitamin D levels than PCOS women without any features of metabolic syndrome (33). The systematic review by Krul-Poel et al (37) suggests that there is an inverse relationship between metabolic disturbances in women with PCOS and vitamin D levels. This is consistent with our study, where we show that having greater number of features of metabolic syndrome is negatively associated with vitamin D levels. A double blind placebo controlled randomized controlled trial (RCT) which studied the effect of vitamin D supplementation on cardiovascular risk factors found that there was no effect of vitamin D supplementation on IR in 50 women with PCOS (39). However, vitamin D supplementation in this trial did improve some cardiovascular risk factors including cholesterol, triglyceride and very low density lipoprotein (VLDL), but not HDL or LDL cholesterol (23). Larger RCTs are needed to further explore the impact of vitamin D supplementation on cardiovascular risk factors.

Limitations of the study include a relatively small sample size. However, in order to detect the difference between lean and overweight women with PCOS, we required 19 participants in each group with a power 97% and alpha of 0.05. Also, this is cross-sectional analysis and demonstrates association between variables. Both prospective studies and clinical trials supplementing vitamin D are needed to prove causality. Patients and controls are not age matched, yet this is adjusted for in the statistical analyses. Commercial immunoassays for vitamin D may produce variable results and tandem mass spectrometry is increasingly the

gold standard test that is used to measure vitamin D. The strengths of this study include the use of strict criteria to define PCOS and non-PCOS women and gold-standard techniques to measure IR and body composition.

In conclusion, our data show that overweight PCOS women had lower vitamin D levels than overweight control women. Vitamin D correlated with IR and adiposity on gold standard measures including BMI, percentage body fat and abdominal visceral fat. Finally, vitamin D was associated with IR in the PCOS group independent of body fat percentage, but not in the non-PCOS group. This suggests that vitamin D may have a role in IR in PCOS; however further exploration in future mechanistic studies is needed. In addition, large-scale intervention studies are needed to explore the preventative potential of vitamin D supplementation on development of IR in PCOS.

Conflict of interest:

The authors have nothing to disclose.

Acknowledgements

We would like to thank Dr Samantha Hutchinson for her involvement in collecting the clamp data for the overweight/obese participants and Mr Sanjeeva Ranasinha for assistance with statistical analysis.

Funding: This work was supported as an investigator initiated trial funded by a competitive grant from Monash University. Barbora de Courten, Helena J Teede and Nigel K Stepto are supported by research grants from the National Health and Medical Research Council of Australia (NHMRC) and Helena J Teede and Jacqueline Boyle are also NHMRC Practitioner

Fellows. Nigel K Stepto was also supported by the Australian Government's Collaborative Research Network Funding Scheme. Cheryce Harrison is a National Heart Foundation (NHF) Postdoctoral Research Fellow. Anju E Joham is a NHMRC postgraduate scholarship holder. Samantha Cassar is supported by an Australian Postgraduate Award through Victoria University.

Author contributions:

AEJ: concept and design, recruited participants, data analysis, interpretation of results, drafted manuscript, revised manuscript for important intellectual content

HJT: development of the methodology, revised manuscript for important intellectual content

SC: recruited participants, revised manuscript for important intellectual content

NKS: development of the methodology, revised manuscript for important intellectual content

BJS: acquisition of data, revised manuscript for important intellectual content

CLH: recruited participants, revised manuscript for important intellectual content

JB: revised manuscript for important intellectual content

BdC: assisted with data analysis and interpretation of results, revised manuscript for important intellectual content

All authors were involved in writing the paper and had final approval of the submitted and published versions.

REFERENCES

1. Mousa A, Naderpoor N, Teede HJ, de Courten MP, et al. Vitamin D and Cardiometabolic Risk Factors and Diseases. *Minerva Endocrinol*. 2015.

2. Holick MF, Chen TC. Vitamin D deficiency: a worldwide problem with health consequences. *Am J Clin Nutr*. 2008, *87*(4):1080S-6S.

3. Ku YC, Liu ME, Ku CS, Liu TY, et al. Relationship between vitamin D deficiency and cardiovascular disease. *World journal of cardiology*. 2013, *5*(9):337-46.

4. Pilz S, Kienreich K, Rutters F, de Jongh R, et al. Role of vitamin D in the development of insulin resistance and type 2 diabetes. *Curr Diab Rep*. 2013, *13*(2):261-70.

5. Holick MF. Vitamin D status: measurement, interpretation, and clinical application. *Ann Epidemiol*. 2009, *19*(2):73-8.

6. Daly RM, Gagnon C, Lu ZX, Magliano DJ, et al. Prevalence of vitamin D deficiency and its determinants in Australian adults aged 25 years and older: a national, population-based study. *Clin Endocrinol (Oxf)*. 2012, *77*(1):26-35.

7. Liu E, Meigs JB, Pittas AG, McKeown NM, et al. Plasma 25-hydroxyvitamin d is associated with markers of the insulin resistant phenotype in nondiabetic adults. *J Nutr*. 2009, *139*(2):329-34.

8. Chonchol M, Scragg R. 25-Hydroxyvitamin D, insulin resistance, and kidney function in the Third National Health and Nutrition Examination Survey. *Kidney Int*. 2007, *71*(2):134-9.

9. Mattila C, Knekt P, Mannisto S, Rissanen H, et al. Serum 25-hydroxyvitamin D concentration and subsequent risk of type 2 diabetes. *Diabetes Care*. 2007, *30*(10):2569-70.

10. Chiu KC, Chu A, Go VL, Saad MF. Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction. *Am J Clin Nutr*. 2004, *79*(5):820-5.

11. von Hurst PR, Stonehouse W, Coad J. Vitamin D supplementation reduces insulin resistance in South Asian women living in New Zealand who are insulin resistant and vitamin D deficient - a randomised, placebo-controlled trial. *Br J Nutr*. 2010, *103*(4):549-55.

12. March WA, Moore VM, Willson KJ, Phillips DI, et al. The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. *Hum Reprod*. 2010, *25*(2):544-51.

13. The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril*. 2004, *81*(1):19-25.

14. Diamanti-Kandarakis E, Papavassiliou AG. Molecular mechanisms of insulin resistance in polycystic ovary syndrome. *Trends Mol Med*. 2006, *12*(7):324-32.

15. Legro RS, Kunselman AR, Dodson WC, Dunaif A. Prevalence and Predictors of Risk for Type 2 Diabetes Mellitus and Impaired Glucose Tolerance in Polycystic Ovary Syndrome: A Prospective, Controlled Study in 254 Affected Women. *J Clin Endocrinol Metab*. 1999, *84*(1):165-8.

16. Stepto NK, Cassar S, Joham AE, Hutchison SK, et al. Women with polycystic ovary syndrome have intrinsic insulin resistance on euglycaemic-hyperinsulaemic clamp. *Hum Reprod*. 2013, *28*(3):777-84.

17. Wehr E, Pilz S, Schweighofer N, Giuliani A, et al. Association of hypovitaminosis D with metabolic disturbances in polycystic ovary syndrome. *Eur J Endocrinol*. 2009, *161*(4):575-82.

18. Patra SK, Nasrat H, Goswami B, Jain A. Vitamin D as a predictor of insulin resistance in polycystic ovarian syndrome. *Diabetes & metabolic syndrome*. 2012, *6*(3):146-9.

19. Yildizhan R, Kurdoglu M, Adali E, Kolusari A, et al. Serum 25-hydroxyvitamin D concentrations in obese and non-obese women with polycystic ovary syndrome. *Arch Gynecol Obstet*. 2009, *280*(4):559-63.

20. Muscogiuri G, Policola C, Prioletta A, Sorice G, et al. Low levels of 25(OH)D and insulin-resistance: 2 unrelated features or a cause-effect in PCOS? *Clin Nutr*. 2012, *31*(4):476-80.

21. Asemi Z, Foroozanfard F, Hashemi T, Bahmani F, et al. Calcium plus vitamin D supplementation affects glucose metabolism and lipid concentrations in overweight and obese vitamin D deficient women with polycystic ovary syndrome. *Clin Nutr*. 2014.

22. Carmina E. Cardiovascular risk and events in polycystic ovary syndrome. *Climacteric*. 2009, *12 Suppl 1*:22-5.

23. Rahimi-Ardabili H, Pourghassem Gargari B, Farzadi L. Effects of vitamin D on cardiovascular disease risk factors in polycystic ovary syndrome women with vitamin D deficiency. *J Endocrinol Invest*. 2013, *36*(1):28-32.

24. Group REACW. Revised 2003 consensus on diagnostic criteria and long term health risks related to polycystic ovary syndrome. *Fertility and Sterility*. 2004, *81*(1):19-25.

25. Hutchison SK, Stepto NK, Harrison CL, Moran LJ, et al. Effects of Exercise on Insulin Resistance and Body Composition in Overweight and Obese Women with and without Polycystic Ovary Syndrome. *J Clin Endocrinol Metab*. 2011, *96*(1):E48-56.

26. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol*. 1979, *237*(3):E214-23.

27. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. 2011, *96*(7):1911-30.

28. Cussons AJ, Watts GF, Burke V, Shaw JE, et al. Cardiometabolic risk in polycystic ovary syndrome: A comparison of different approaches to defining the metabolic syndrome. *Hum Reprod*. 2008, *23*(10):2352-8.

29. Prentice A. Vitamin D deficiency: a global perspective. *Nutr Rev*. 2008, *66*(10 Suppl 2):S153-64.

30. Thomson RL, Spedding S, Buckley JD. Vitamin D in the aetiology and management of polycystic ovary syndrome. *Clin Endocrinol (Oxf)*. 2012, *77*(3):343-50.

31. Gambineri A, Pelusi C, Vicennati V, Pagotto U, et al. Obesity and the polycystic ovary syndrome. *Int J Obes*. 2002, *26*:883-96.

32. Yildizhan R, Kurdoglu M, Adali E, Kolusari A, et al. Serum 25-hydroxyvitamin D concentrations in obese and non-obese women with polycystic ovary syndrome. *Arch Gynecol Obstet*. 2009, *280*(4):559-63.

33. Wehr E, Pilz S, Schweighofer N, Giuliani A, et al. Association of hypovitaminosis D with metabolic disturbances in polycystic ovary syndrome. *Eur J Endocrinol*. 2009, *161*(4):575-82.

34. Li HW, Brereton RE, Anderson RA, Wallace AM, et al. Vitamin D deficiency is common and associated with metabolic risk factors in patients with polycystic ovary syndrome. *Metabolism*. 2011, *60*(10):1475-81.

35. Lagunova Z, Porojnicu AC, Lindberg F, Hexeberg S, et al. The dependency of vitamin D status on body mass index, gender, age and season. *Anticancer Res*. 2009, *29*(9):3713-20.

36. Scragg R, Sowers M, Bell C. Serum 25-hydroxyvitamin D, diabetes, and ethnicity in the Third National Health and Nutrition Examination Survey. *Diabetes Care*. 2004, *27*(12):2813-8.

37. Krul-Poel YH, Snackey C, Louwers Y, Lips P, et al. The role of vitamin D in metabolic disturbances in polycystic ovary syndrome: a systematic review. *Eur J Endocrinol*. 2013, *169*(6):853-65.

38. Pittas AG, Chung M, Trikalinos T, Mitri J, et al. Systematic review: Vitamin D and cardiometabolic outcomes. *Ann Intern Med*. 2010, *152*(5):307-14.

39. Ardabili HR, Gargari BP, Farzadi L. Vitamin D supplementation has no effect on insulin resistance assessment in women with polycystic ovary syndrome and vitamin D deficiency. *Nutrition research (New York, NY)*. 2012, *32*(3):195-201.

Figure 1: Relationship of Vitamin D to adiposity measures and insulin resistance

A Relationship of vitamin D to body mass index (BMI) in PCOS group, $R^2 = 0.24$, p=0.001

B Relationship of vitamin D to body mass index (BMI) in control group, $R^2 = 0.04$, p=0.26

C Relationship of vitamin D to fat mass in PCOS group, $R^2 = 0.29$, p<0.001

D Relationship of vitamin D to fat mass (BMI) in control group, $R^2 = 0.04$, p=0.27

E Relationship of vitamin D to abdominal visceral fat in PCOS group, $R^2 = 0.29$, p<0.001

F Relationship of vitamin D to abdominal visceral fat in control group, $R^2 = 0.09$, p=0.09

G Relationship of vitamin D to glucose infusion rate (GIR) in PCOS group, $R^2 = 0.22$, p=0.002

H Relationship of vitamin D to glucose infusion rate (GIR) in control group, $R^2 = 0.005$, p=0.71

Figure 2: Relationship between vitamin D and number of metabolic risk factors

Mean plasma vitamin D level ± standard deviation